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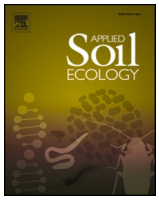

Review Article

Earthworm inoculation in degraded soils: A meta-analysis

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ABSTRACT

Earthworms are key soil organisms involved in several soil functions. Earthworm inoculation can be used in the context of the agroecological transition and of Nature-based solutions to produce food while restoring soil fertility. In this meta-analysis we wanted to identify the earthworm inoculation parameters (density of the inoculated earthworms, time between inoculation and sampling, presence/absence of physical barriers, and number of species inoculated) that lead to a successful intervention (earthworm abundance and biomass increase).

For this quantitative analysis of earthworm inoculation success, we exhaustively reviewed the literature using the ISI Web of Knowledge with the "All Databases" option. We selected articles on field experiments that compared earthworm abundance or biomass in the inoculated area and in a clearly defined control area (same sampling period). We excluded articles on laboratory studies, vermicomposting, and non-intentional earthworm introduction.

Based on the 25 studies included in the corpus, the meta-analysis showed an increase in the abundance of inoculated earthworm species (4.48-fold) and of the whole earthworm community (1.55-fold). When >150 individuals m^{-2} were inoculated, the abundance of the inoculated earthworm species increased by a factor of 48.0 compared with the control plot. By monitoring the efficiency after at least 2 years, the increase in inoculated species abundance reached a factor of 37.0. Conversely, in short-term studies (<1 year), this increase factor was not significant and was reduced to 1.9. Multi-species inoculations (i.e., different ecological categories of earthworms) could be more efficient (inoculated species abundance and total community biomass increases by a factor of 30.3 and 3.6, respectively). In a soil restoration perspective, an efficient inoculation must be designed in function of the targeted soil functions.

To perform a successful inoculation, we recommend to inoculate >150 individuals m^{-2}, preferably of different species, and to assess their establishment after a relatively long interval (>2 years after inoculation).

1. Introduction

Agricultural soils are increasingly degraded (e.g., organic matter depletion, contamination, compaction), mainly due to the intensive agricultural practices used for food production for many decades such as intensive tillage, bare soil or fertilizer application (Calatrava et al., 2011; de Valença et al., 2017). Currently, 33 % of soils worldwide are considered to be moderately to highly degraded (FAO and ITPS, 2015). Besides climate change, land degradation has negative effects on crop yields, with an estimated reduction between 10 and 50 % by 2050 (Scholes et al., 2018). Land degradation also has negative effects on soil biodiversity (Baude et al., 2019; Gregory et al., 2015), with detrimental effects on several taxa, such as beetles (Müller et al., 2022), nematodes (Wu et al., 2017) and earthworms (Li et al., 2021).

Earthworms are soil ecosystem engineers (Jones et al., 1994) that contribute to the soil organic matter dynamics (Lavelle and Martin, 1992; Lemtiri et al., 2014) and the soil nutrient availability for plants and other soil organisms (Medina-Sauza et al., 2019; Xiao et al., 2018). They also increase soil porosity and consequently water transfers through the creation of macropores of different shape and size, according to the species (Capowiez et al., 2015). A meta-analysis (van Groenigen et al., 2015) showed an average increase by 25 % in crop yields and by 23 % in aboveground plant biomass when earthworms are present in agroecosystems.

To address the growing demands for food production caused by the global demographic growth, stakeholders (e.g., policy makers, farmers)

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are looking for innovative and sustainable solutions to maintain the current yields while enhancing soil biodiversity. Many calls have been made for an agroecological transition to switch from conventional to more sustainable food production systems (Chantre and Cardona, 2014; Sachet et al., 2021). Agricultural practices, such as intercropping (Schmidt et al., 2001), organic matter inputs (Pérès et al., 1998), no-tillage (Brown et al., 2003) and longer crop rotations (Rodríguez et al., 2020), have positive effects on soil biodiversity and fertility. Earthworm inoculations also can be used to support these changes, enhance fertility, and restore degraded soils (Butt et al., 1997; Ratsiatosika et al., 2021).

Earthworm inoculation was first tested by a New Zealander farmer who in the early 1950s, by transplanting soil turves containing *Aporrectodea caliginosa* (exotic species in New Zealand), related the earthworm abundance to the fertility of his soil (Nielson, 1951). Since then, scientists have tested different earthworm inoculation techniques to restore degraded soils (Butt et al., 1995; Piearce et al., 2003) and observed positive impacts of earthworm inoculation on different soil components. For instance, in surface mines of South Wales, soil porosity in the first twenty centimeters was increased 6 years after earthworm inoculation (Marashi and Scullion, 2004). Similarly, macroporosity was increased after the inoculation of *Lumbricus terrestris* in a unploughed clay loam soil of a Southwest Québec forest (Brais et al., 1989). However, to observe and maintain these positive effects, it is necessary to allow the establishment of earthworm populations or communities in the inoculated fields.

In this meta-analysis we wanted to identify the earthworm inoculation parameters (density of the inoculated earthworms, time between inoculation and sampling, presence/absence of physical barriers, and number of species inoculated) that lead to a successful intervention (earthworm abundance and biomass). We included studies in which earthworm abundance or biomass (i.e., the endpoints) were quantified in inoculated and control (without earthworm inoculation) plots at different time points after inoculation. We hypothesized that i) after earthworm inoculation, earthworm abundance and biomass increase are mainly due to the reproduction of inoculated individuals; and ii) the abundance and biomass measured in the inoculated fields increase proportionally with the number of inoculated individuals (i.e., here called inoculation density). We also hypothesized that i) interactions between earthworm species from different ecological categories (multi-species versus mono-species inoculation) may favor the establishment of the different populations in the field; and ii) physical barriers might allow retrieving more earthworms at the inoculation point because some authors used them to prevent earthworm dispersion during the experiment and to maximize their effects on various soil components. Lastly, we collected data on the overall earthworm community (i.e., inoculated plus native species) after the inoculation experiments to determine whether the inoculated species replaced or increased the native earthworm community (if present). On the basis of the meta-analysis findings, we then made recommendations for optimizing these Nature-based initiatives, particularly concerning the earthworm species and densities to be inoculated.

2. Materials and methods

2.1. Literature search

The literature search was carried out in the ISI Web of Knowledge with the "All Databases" option and using the following key words: (earthworm* or lumbric* or aporrect* or eisen* or dendrob* or allolobophor* or octolas* or dendrodril* or diporodril* or eophil* or helodril* or kritodril* or octodril* or prosellodril* or scherothec* or satchell* or proctodril* or orodril* or postandril* or perelia* or andrei* or fetid* or foetid*) and (inoculat* or introduc* or restor* or bioremediat* or biostimulat* or biofertilizat* or revitalizat*) in the title. The term 'regenerat*' was also tested, but as 95 % of the retrieved publications were on epidermal regeneration in earthworms, this word was not considered in the final formula.

The search concerned papers published until December 2022. It was restricted to titles because a topic-based search with the chosen formula yielded 5604 references, most of which were out of topic. A total of 287 references were identified. Their titles and abstracts were studied to select relevant articles. To complete the dataset, the bibliography of the selected articles was scanned to identify other publications on the topic of interest. Most of the papers were found with the word "earthworm*" (92 % of the total corpus). We decided to include some genius names to complete the search and we used the Lumbricidae family as they are the most commonly studied. Articles on field studies that compared earthworm abundance or biomass in the inoculated area and in a clearly defined control area (same sampling period) were selected. Laboratory studies were excluded to focus only on field experiments. In order to address the main objective of this study (i.e., effect of intentional earthworm introduction on earthworm density for soil restoration objective), studies on vermicomposting and non-intentional earthworm introduction also were excluded from the corpus. Studies with samples collected at different times in the inoculated and control plots were excluded because seasonality is an important factor in driving earthworm abundance (Tondoh, 2006). Articles only on earthworm dispersal from the inoculation point also were excluded because they did not assess abundance at the inoculation point. In some cases, the highest distance from the inoculation point was considered as the control for this literature review if the risk of contamination over time was low (minimum 6 m per year) and the data of this distant plot do not suggest any contamination. This literature search allowed selecting 25 references (Supplementary Material S1).

2.2. Data extraction

From each of the 25 selected articles, the earthworm abundance and biomass in the control (not inoculated) and inoculated plots were extracted. Raw data (i.e., in tables or in appendices) were preferentially used. When data were presented in figures, they were extracted using the Image J software. Adults, sub-adults, juveniles and cocoons were all considered as individuals in our analysis. Some authors gave a detailed description of the number of sub-adults, juveniles and cocoons recorded (Curry and Boyle, 1995; Frazão et al., 2019; Garnsey, 1994; Gilot-Villenave et al., 1996). In those cases, all life stages were considered in the analysis, using adult abundance ratio when necessary. When this adult abundance ratio could not be used due to presentation form of the data (Forey et al., 2018), juveniles or cocoons were only considered in the community analysis that did not require a precise species identification. Standard deviation values were extracted when available, or calculated using the standard error and the number of replicates with the following formula:

$$SD = SE \times \sqrt{N}$$

where N is the number of replicates, SD the standard deviation, and SE the standard error.

If the standard deviation or standard error was not given, the maximum variation coefficient of the endpoint (earthworm abundance or biomass) in the obtained dataset (inoculated earthworms or earthworm community) was applied to the observation to assign it a conservative standard deviation. For each selected article, the following data were recorded: author, year, endpoint (abundance, biomass) in control and inoculated plots (for inoculated earthworms and earthworm community) with their standard deviation, number of replicates, number of inoculated species, inoculation density, physical constraint (i.e., use of a physical barrier or not), and time between inoculation and sampling. Other information such as inoculation country, climate (using Köppen-Geiger climate classification of Beck et al., 2018), earthworm origin (field collected or obtained from breeding systems) as well as the

main management practices used are detailed for each observation in Appendix S1.

When several inoculations were performed in the same study (Golli, 2015), the numbers of earthworms used at each inoculation were totaled and the date of the last inoculation was considered as the inoculation date. If sampling was performed between inoculations, the inoculations before and after sampling were considered as different inoculations and thus different lines were added in the dataset. In some studies (Blanchart et al., 2004; Gilot, 1997), the native earthworm community was removed from the experimental plots by initially killing earthworms with a toxic chemical (e.g., carbofuran). Therefore, for some observations, the number of earthworms in the control (and sometimes in the inoculated plot when the inoculation failed) was 0 or < 1. For calculation purposes, the value of 1 was added to the mean abundance and biomass in the whole database (i.e., in control and inoculated plots), thus maintaining the same ratio between compared plots. Unpublished data given by the authors showed sometimes that they focused only on the inoculated species. In those cases, the paper was not considered for the community analysis.

2.3. Categories

To test the different hypothesis, four categories were used (1) earthworm inoculation density; (2) time interval between inoculation and sampling; (3) presence or absence of a physical barrier (e.g., geotextile or PVC sheets); and (4) number of earthworm species inoculated (Table 1). When no information was given on a specific category, the study was not considered for that category. For instance, when no information was given on the inoculation density (Stein et al., 1992), the paper was not considered for this category. Concerning the inoculation density, studies were classified in function of the number of earthworms inoculated: (i) <50 individuals m^{-2}, (ii) between 50 and 150 individuals m^{-2}, and (iii) >150 individuals m^{-2}. All life stages were considered as individuals for the density calculation. When the inoculation density was in g m^{-2} (Golli, 2015), this value was transformed using the mean biomass of the inoculated species given in the article or in one case (Gilot, 1997), in the other corpus article by the same author using the same species. Concerning the interval between inoculation and sampling, three time lengths were considered: (i) <1 year, (ii) between 1 and 2 years, and (iii) >2 years. Concerning the number of inoculated species, two options were considered: (i) mono-species (one species) and (ii) multi-species (more than one species) inoculation. Except for one study (Golli, 2015), multi-species inoculation involved species from different main ecological categories (i.e., epigeic, endogeic, anecic).

2.4. Effect size calculation

The effect size was calculated for all the observations collected in the dataset (Supplementary Material S1) using the logarithm of the response ratio (Hedges et al., 1999):

$$ln\,R = ln\,(I/C)$$

where I and C are the mean values of the inoculated plot and control plot, respectively.

The pooled data variance was calculated with the following formula (Hedges and Olkin, 1985):

$$var. = SD_I{}^2/(N_I \times I^2) + SD_C{}^2/(N_C \times C^2)$$

where SD_I and SD_C are the standard deviations of the inoculated plot and control plot, respectively, and N_I and N_C are the number of replicates of the inoculated plot and control plot, respectively.

The mean effect size was calculated using the metafor package on the R software; observations were weighted using the inverse-variance method (or median weight for null variance) where the weight is the inverse of the pooled variance (Hedges and Olkin, 1985). To convert the mean effect size in an increase or decrease factor, a transformation with the exponential function was used.

For both abundance and biomass data (i.e., line), three different models were tested with one of the following random effects: 'Author' or 'Line' or 'Author + Line'. The models with the random effects on 'Author + Line' were selected because they always presented the lowest Akaike Information Criterion (AIC) value.

Publication bias was visualized with a Doi plot-based method followed by quantification using the LFK index (Furuya-Kanamori et al., 2018), a new approach with higher sensitivity than the widely used Egger's regression method. When a variance was equal to 0, we attributed to these data the lowest non-null variance in the database for this calculation. If publication bias was detected, a failsafe number was calculated using the Orwin approach to determine the number of studies that averaged the number of null result needed to reach the target effect size (Orwin, 1983). In this meta-analysis the target effect size was the default value equal to half of the observed effect size.

3. Results

3.1. Data description

Among the 25 publications included in the analysis, the oldest was published in 1969 and 13 (52 %) between 1992 and 1999 (Supplementary Material S1). It is worth specifying that the majority of the corpus is composed of studies conducted in temperate climate, with only 20 % (5 studies out of 25) in tropical climates. Regarding earthworm origin, inoculated individuals were mostly collected in the surrounded fields and in some rare cases, using commercial or non-commercial breeding. The important number of missing information and the non-equilibrium between field collected and breeding origins made irrelevant the meta-analysis calculation on this parameter. Data on the

Table 1
Number of observations (and publications) for each category in the meta-analysis dataset.

Inoculation parameter	Category	Inoculated earthworm abundance	Inoculated earthworm biomass	Earthworm community abundance	Earthworm community biomass
Density (individuals. m^{-2})	< 50	59 (7)	36 (4)	47 (7)	36 (4)
	50–150	49 (9)	13 (4)	49 (9)	8 (3)
	> 150	28 (7)	8 (3)	21 (5)	11 (4)
Time interval (years)	< 1	58 (10)	35 (7)	45 (7)	34 (6)
	1–2	53 (6)	8 (2)	47 (6)	6 (1)
	> 2	28 (10)	14 (5)	27 (9)	15 (5)
Physical barrier	Yes	62 (9)	19 (5)	59 (8)	14 (4)
	No	77 (14)	38 (5)	60 (12)	41 (6)
Species number	Mono-species	111 (14)	46 (7)	94 (12)	41 (6)
	Multi-species	28 (12)	11 (5)	25 (10)	14 (6)
TOTAL		139 (23)	57 (10)	119 (20)	55 (10)

inoculated earthworm abundance were more frequently found than data on the inoculated earthworm biomass: 139 (in 23 publications) versus 57 (in 10 publications) side by side comparisons (i.e., lines or observations), respectively (Table 1). A similar difference was observed for the earthworm community abundance and biomass: 119 (in 20 publications) and 55 (in 10 publications) side by side comparisons, respectively (Table 1). The single paper monitoring earthworm community biomass between 1 and 2 years after earthworm inoculation will prevent generic interpretation for this category (Table 1). Sampling at <2 years post-inoculation was more frequent than >2 years. Data on inoculation with multi-species were four times less frequent than data on inoculation with mono-species. Lastly, more data were collected with inoculation density < 50 individuals m^{-2} than >150 individuals m^{-2}.

Publication bias was detected for the abundance and biomass of inoculated earthworms (Supplementary Material S2). The failsafe numbers for these two endpoints were 139 and 57, respectively. The failsafe number of inoculated earthworm abundance was higher than the one of biomass, with more studies needed to balance the mean effect size. There was no publications bias for the earthworm community abundance and biomass.

3.2. Abundance and biomass of the inoculated earthworms

The mean effect size calculated by taking into account all observations collected in the dataset revealed a positive effect of inoculation initiatives on the inoculated earthworm abundance (Fig. 1a), as indicated by the increase factor value of 4.48 (i.e., $e^{1.50}$). Conversely, the inoculated earthworm biomass did not significantly increase after inoculation (Fig. 1b).

For both endpoints (abundance and biomass) and categories, all calculated mean effect sizes were positive (lowest value = 0.17, for the inoculated earthworm biomass with intermediate inoculation density), although not always significantly different from 0 (Fig. 2). This indicates that earthworm abundance and biomass were higher in inoculated areas than in control plots. However, due to the 95 % confidence intervals (CIs), these effects were not always significant.

Specifically, only inoculation densities >150 individuals m^{-2} significantly increased the inoculated earthworm abundance (mean increase factor of 48.0 compared with control plot). Physical barriers did not have any effect on the abundance and biomass of the inoculated earthworms. Concerning the interval after the inoculation, the abundance of the inoculated species increased, compared with control plots (no inoculation) when this time was longer than 2 years. Between 1 and 2 years, only the biomass of the inoculated earthworms increased compared with the control plots. Lastly, the abundance of inoculated earthworms significantly increased by a factor of 30.3 after multi-species inoculation, in contrast to mono-species inoculation (Fig. 2).

3.3. Abundance and biomass of the earthworm community

The mean effect size calculated by taking into account all observations in the dataset revealed a positive effect of the inoculation initiatives on the abundance and biomass of the total earthworm community, with increase factors of 1.55 and 2.23, respectively (Fig. 3a-b).

For all endpoints and categories, the mean effect sizes were positive, but not always significant. The lowest value (0.19) for earthworm community abundance was obtained for inoculation using <50 individuals m^{-2} (Fig. 4).

High inoculation density (>150 individuals m^{-2}) significantly increased the earthworm community abundance and biomass by a mean factor of 1.9 and 4.9, respectively. Similarly, the presence of physical barriers significantly increased the community abundance. A significant increase in community biomass was observed when sampling was observed >2 years post-inoculation. No significant increase in community abundance was observed for the different time intervals. Lastly, the biomass of the earthworm community significantly increased by a factor of 3.6 after multi-species inoculation, in contrast to mono-species inoculation (Fig. 4).

4. Discussion

This meta-analysis showed that earthworm inoculation can be an efficient strategy for increasing the abundance and/or biomass of the inoculated individuals and also of the total community. These positive effects were observed especially after 1 or 2 years, for inoculation initiatives with >150 individuals m^{-2}. Although the increase in earthworm abundance and biomass was not always significant, the calculated mean effect sizes were all positive. This indicates that generally, adding some earthworm species will not harm the earthworm communities. The dataset variance, which is common when earthworms are sampled in the field, may explain these non-significant effects.

Our findings indicate that the inoculation density is a crucial parameter for successful earthworm inoculations. A density > 150 individuals m^{-2} efficiently increased earthworm abundance in soils. This

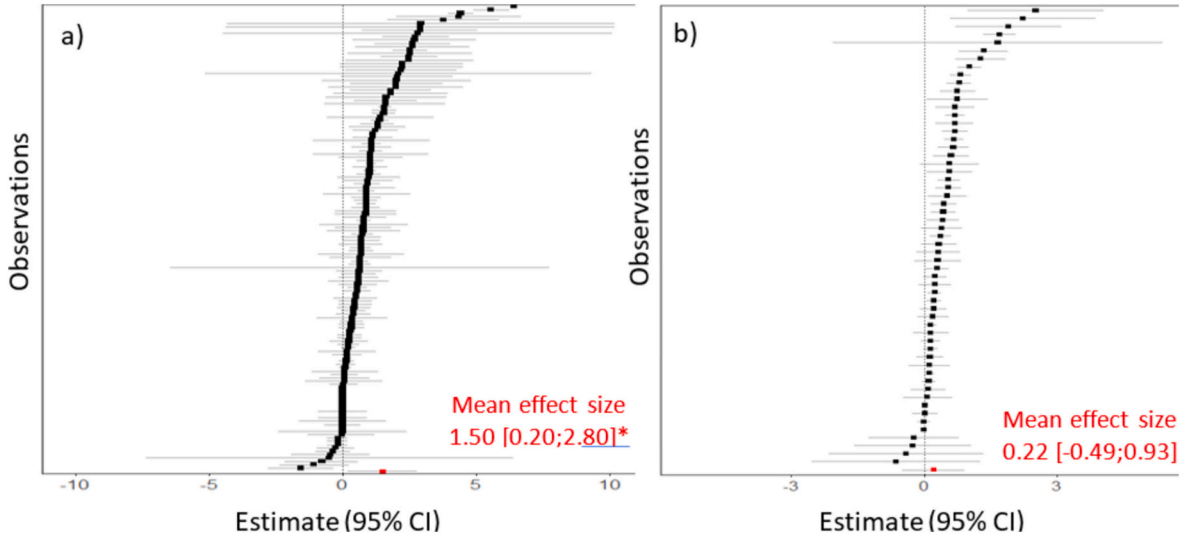

Fig. 1. Natural logarithm of the ratio of inoculated earthworm a) abundance and b) biomass at the inoculated plots vs the control plots. Red, mean effect size [95 % confidence intervals, CI]. Each line corresponds to an observation (Supplementary Material S1). The error bars correspond to the 95 % CI and * indicates a significant effect. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

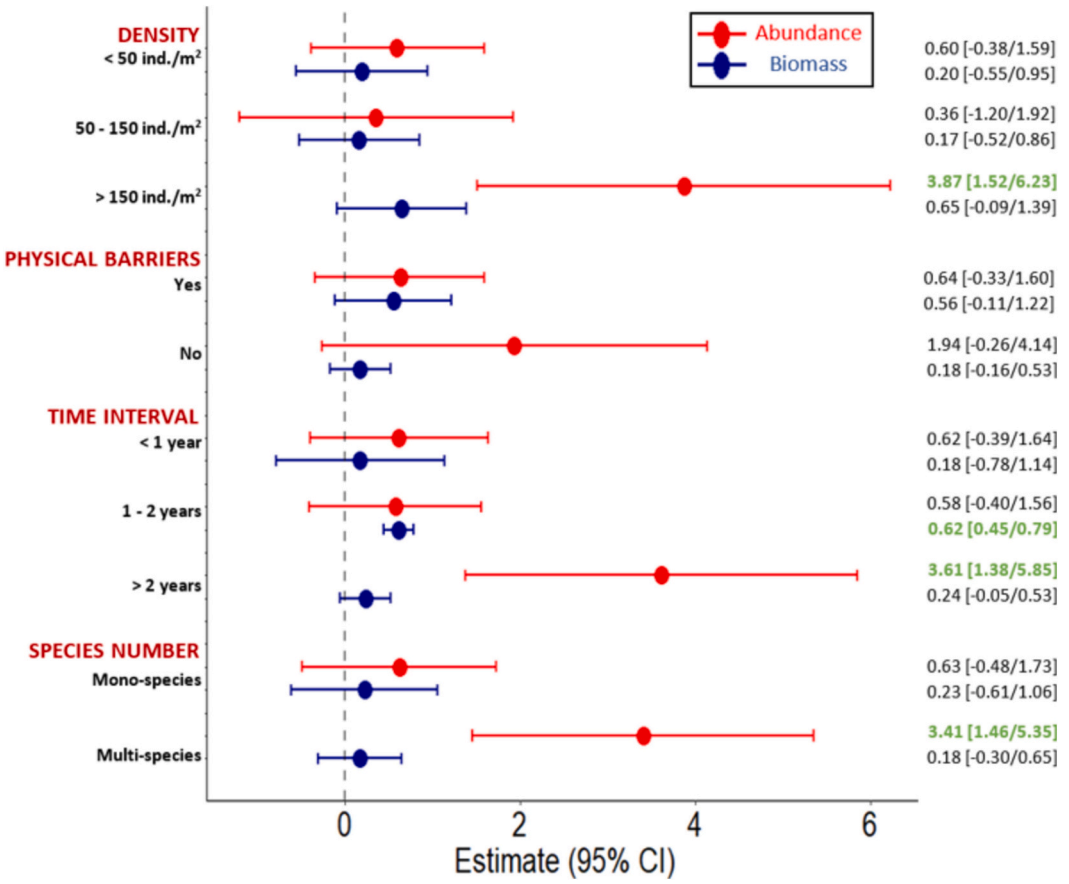

Fig. 2. Mean effect size [95 % CI] of inoculated earthworm abundance (red) and biomass (blue) after an inoculation initiative according to the four categories analyzed. Green, significant mean effect size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

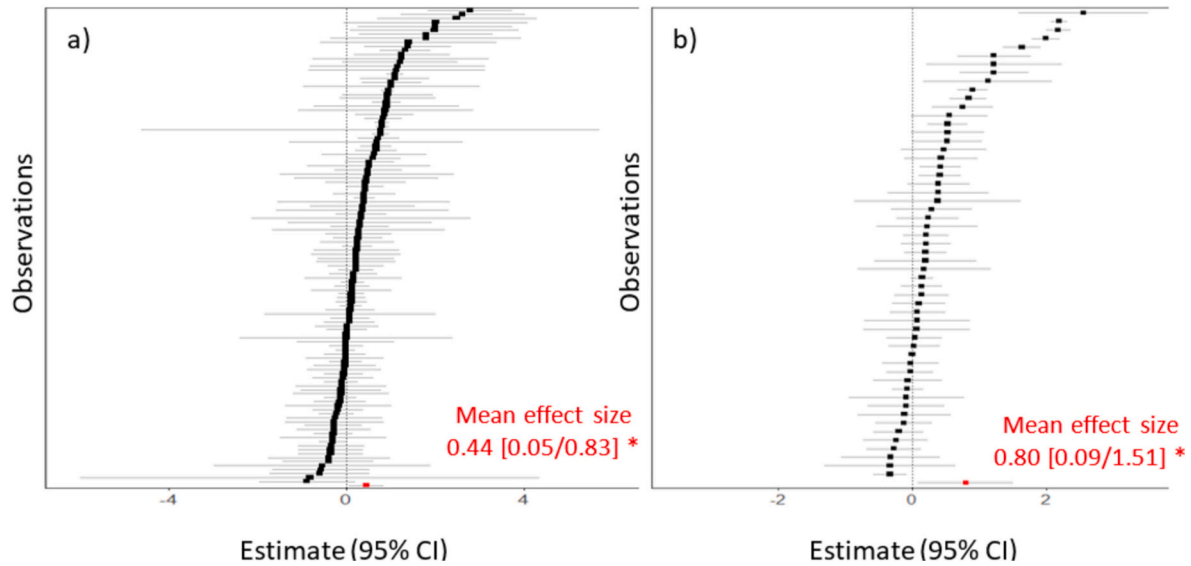

Fig. 3. Natural logarithm of the ratio of total earthworm community a) abundance and b) biomass at the inoculated plots vs control plots. Red, mean effect size [95 % CI]. Each line corresponds to an observation (Supplementary Material S1). The error bars correspond to the 95 % CI and * indicates a significant effect. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

can be explained by the high mortality rate of the inoculated earthworms. Survival of the initial inoculum is one of the essential steps in the inoculation initiative success (Butt, 1999). Their handling to prepare the inoculum can be harmful. Moreover, the adaptation to a new habitat and the interactions with existent earthworm communities can be stressful for the inoculated earthworms. Some of the inoculated earthworms may be negatively affected or even die if the conditions are not favorable: important bird predation, drought or agricultural practices such as soil

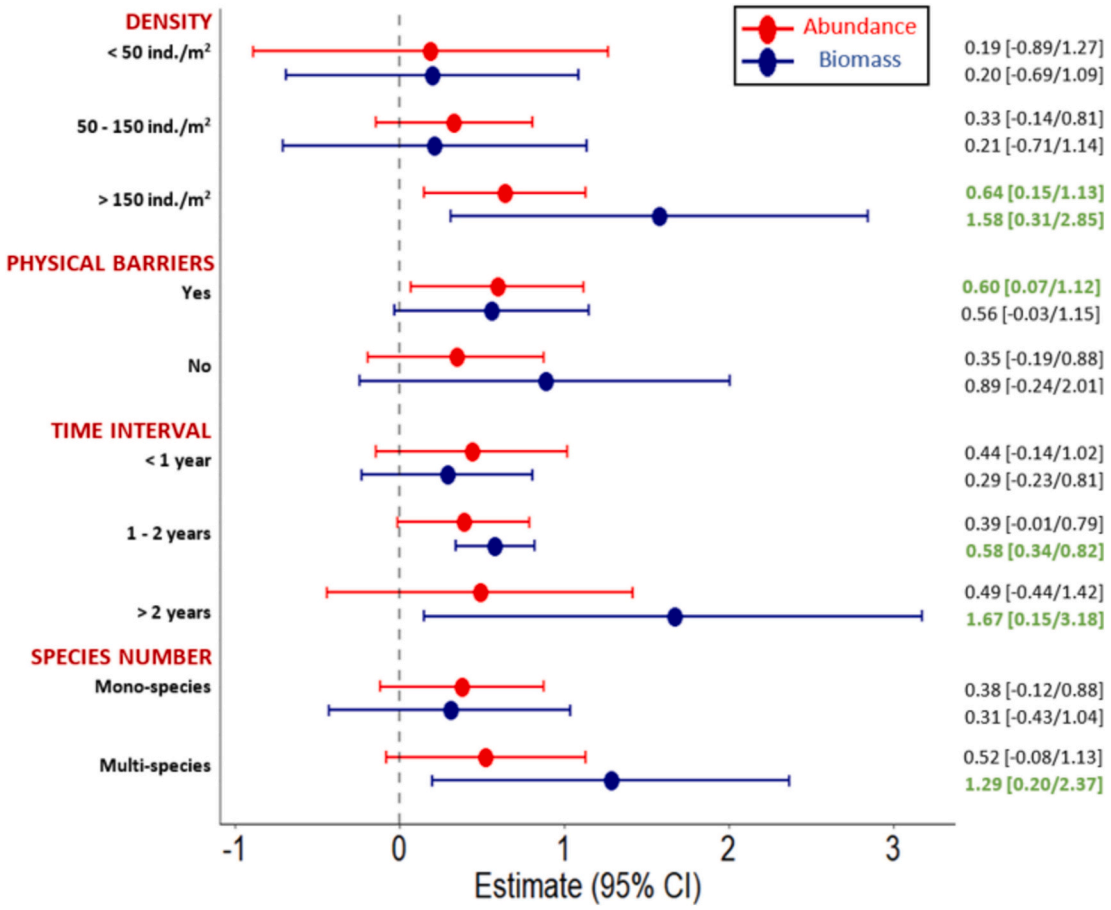

Fig. 4. Mean effect size [95 % CI] of earthworm community abundance (red) and biomass (blue) after an inoculation initiative according to the four categories analyzed. Green, significant mean effect size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tillage (Butt et al., 1997; Nuutinen et al., 2011). For instance, earthworm inoculation timing was mentioned to be a driving factor of the survival of inoculated individuals, with the recommendation to inoculate earthworms in UK either in spring or in fall, where soil moisture and temperature are optimal (Butt, 2008). Optimal soil moisture condition was for instance described in some papers of the corpus (e.g., Baker et al., 1999). As survival is proportional to the number of inoculated earthworms, higher inoculation densities increase the chance that earthworm reproduction might help to maintain or increase the populations. However, for soil rehabilitation by earthworm introduction, retrieving about half of the inoculated earthworms at the inoculation point is encouraging also because the individuals may have dispersed from the inoculation point (Nuutinen et al., 2011; Schon et al., 2014).

In order to support earthworm establishment after inoculation in soil restoration perspectives, management practices have to be carefully chosen. For example, almost half of the papers of the corpus have studied organic matter addition, at least in one condition (Supplementary Material S1). These organic matter treatments can be different with for instance the use of chicken and cow manure (van Der Werff et al., 1998), or crop residues such as maize (Gilot-Villenave et al., 1996), rice (Ratsiatosika et al., 2021) and poplar litter (Ampoorter et al., 2011). Lime addition, that was found to be often studied in the corpus (Supplementary Material S1), increased the earthworm density in a forest soil (Robinson et al., 1992). Environmental conditions, especially regarding food availability, are a crucial parameter to consider for long-term earthworm establishment.

The time interval between inoculation and sampling also was important to assess the success of earthworm inoculation initiatives. Our findings indicate that >2 years are required to detect a significant increase in earthworm abundance in the inoculated areas compared with the control areas. A 1-year interval is not enough to overcome the low survival rate of the inoculated earthworms through reproduction. The full life cycle is between 4 and 6 months for *A. caliginosa* (Bart et al., 2018) and 6 months for *L. terrestris* and *Aporrectodea longa* (Butt, 1993). This highlights the importance of long-term assessments in earthworm-based soil rehabilitation initiatives due to the need to allow their reproduction, especially considering the time needed for the rehabilitation of complex biological systems (Ramade, 2020).

Although the inoculation success can be measured directly at the inoculation point, it is important also to assess the earthworm capacity to colonize the site over time. In our dataset, 14 publications did not use any physical barrier around the inoculation point. Among them, only five studies (i.e., 20 % of the total dataset) monitored the dispersion of the inoculated earthworms in addition to their abundance at the inoculation point. At 2 months post-inoculation, van Rhee (1969) reported that *A. caliginosa* had spread up to 3 m, but not L. *terrestris*, in accordance with the very low survival rate of this species at the inoculation point. Transects can be used to monitor earthworm dispersal, especially in long-term studies. Otherwise, earthworms can be directly sampled starting from the inoculation point and then walking away (Stein et al., 1992; van der Werff et al., 1998), or casts at the soil surface can be visually counted (Butt et al., 1999). After 10 years, the highest density of the inoculated species *Allolobophora chlorotica* and *A. longa* in a landfill site in the United Kingdom was observed at 6 and 10 m from the inoculation point, respectively (Butt et al., 2004). The authors determined using a linear regression model based on earthworm density data that

these species could have reached 57 m and 65 m in this period of time, respectively (i.e., 5.7 m and 6.5 m per year). At the same site and using cast observation, *A. longa* was observed at 132 m, highlighting the different results obtained using two migration assessment methods. The dispersion of 6.5 m per year is in accordance with *A. longa* dispersion (5.3 to 12.5 m per year) reported by a long-term study (>20 years) at two inoculated sites (Schon et al., 2014). Similarly, *L. terrestris* was sampled at 60 m from the inoculation point after 13 years (Nuutinen et al., 2011).

In complex systems, interactions between species can be detrimental or beneficial. Intra- and also inter-specific interactions can be positive, negative, or neutral (Uvarov, 2009). Here, we found that the inoculation initiative success was generally higher when different species were inoculated together in the same time. In the included articles, most studies with multi-species inoculation involved species from different ecological categories. One hypothesis could be that interactions of species with different ecology could bring mutual benefits by working complementarily in the soil. Thus, species traits and preferences should be considered in the inoculation design because the species choice is crucial for the inoculation success (Butt, 2011). For instance, *L. terrestris* and *A. longa,* as well as *A. chlorotica* and *A. caliginosa* may compete in field conditions (Edwards and Lofty, 1980). Conversely, epi-anecic species interactions have a positive effect on their individual biomass (Hoeffner et al., 2019). Therefore, it is essential to design the inoculation strategy by taking into account these interactions for optimal inoculation success and positive effects on soil functioning. For example, 2–5 mm aggregates and leaf production increased in a maize crop inoculated with two different endogeic species, *Millsonia anomala* and *Chuniodrilus zielae* (Derouard et al., 1997). In this case, although both species were endogeic, the compacting action of the first was complementary to the decompacting effect of the second. Our meta-analysis showed that the presence of inoculated earthworms positively influences the whole earthworm community; however, earthworm inoculation may also influence other soil taxa that are important for soil functioning. For instance, a negative effect of the inoculated earthworms was highlighted on the abundance of some oribatid species (McLean and Parkinson, 2000) and on the abundance and diversity of collembola (Forey et al., 2018). However, positive effect such as the decrease of the relative abundance of the plant parasitic nematode *Pratylenchus vulnus* was shown after the combined action of earthworm inoculation (*Amynthas corticis*) and cover crop (*Lotus uliginosus*), resulting in a decrease in maize infection (Boyer et al., 1999).

The aim of this meta-analysis was to identify the key methodological parameters for a successful earthworm inoculation. However, when designing an inoculation initiative, it is also crucial to take into account the environmental features of the site (e.g., soil type and texture) and the geographical area because these criteria can influence the earthworm effect on several component such as plant production (van Groenigen et al., 2015). Unfortunately, in our dataset, data were too scarce to perform a relevant analysis on this topic. Moreover, the comparison between laboratory-bred and field collected earthworms could be important because little is known about the introduction of laboratory-bred earthworms and their adaptation to a new environment with fluctuating soil moisture and temperature conditions (Wever et al., 2001).

In our dataset, publication bias could be explained by the low number of negative observations (i.e., observations with a neutral or negative effect of earthworm inoculation on the inoculated earthworms and total community). This general positive effect was expected because we assumed that earthworm addition in a soil could at worst be neutral, without any effect on earthworm abundance or biomass. Additionally, it is very likely that studies with statistically insignificant results are less frequently published (Rockwell et al., 2006). The high data variance resulted often in not significant findings, even for high mean effect sizes (e.g., inoculated earthworm abundance in the absence of a physical barrier). This high variance is mainly linked to our conservative approach (i.e., using the maximum variance of the whole dataset for the studies that did not report any information on variance). In the analysis of the inoculated earthworm abundance, the maximum variation coefficient was attributed to 19.4 % of all observations (27/139 observations). New studies with detailed information on the results and on the methodology used may be very informative.

5. Conclusion

Earthworm inoculation is a promising Nature-based solution to increase earthworm abundance and biomass in soil. It can be used after the ecological diagnosis and if the agricultural practice changes are not sufficient to restore biodiversity. A careful inoculation design is needed to maximize the chance of success and the effects on soil functioning. To optimize the establishment of inoculated earthworm individuals in a soil, we recommend introducing different earthworm species and at high density (>150 individuals m^{-2}), without physical barriers. We also recommend to assess the dispersion (and reproduction) of the inoculated individuals after a long interval after inoculation (> 2 years). Even if before any inoculation initiatives sustainable agricultural practices have to be first adopted, earthworms should be seen as key levers for supporting the agroecological transition because of their positive impact on ecosystem functions (Liu et al., 2019) and biomass production (van Groenigen et al., 2015).

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CRediT authorship contribution statement

Lucas Petit-dit-Grézériat: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Magali Rault:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Cécile Serbource:** Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Céline Pelosi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Ampoorter, E., De Schrijver, A., De Frenne, P., Hermy, M., Verheyen, K., 2011. Experimental assessment of ecological restoration options for compacted forest soils. Ecol. Eng. 37, 1734–1746. https://doi.org/10.1016/j.ecoleng.2011.07.007.

Baker, G.H., Carter, P.J., Barrett, V.J., 1999. Survival and biomass of exotic earthworms, *Aporrectodea spp.* (Lumbricidae), when introduced to pastures in South-Eastern Australia. Aust. J. Agr. Res. 50, 1233–1245.

Bart, S., Amossé, J., Lowe, C.N., Mougin, C., Péry, A.R.R., Pelosi, C., 2018. *Aporrectodea caliginosa*, a relevant earthworm species for a posteriori pesticide risk assessment: current knowledge and recommendations for culture and experimental design. Environ. Sci. Pollut. Res. 25, 33867–33881. https://doi.org/10.1007/s11356-018-2579-9.

Baude, M., Meyer, B.C., Schindewolf, M., 2019. Land use change in an agricultural landscape causing degradation of soil based ecosystem services. Sci. Total Environ. 659, 1526–1536. https://doi.org/10.1016/j.scitotenv.2018.12.455.

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. Sci. Data 5, 180214. https://doi.org/10.1038/sdata.2018.214.

Blanchart, E., Albrecht, A., Chevallier, T., Hartmann, C., 2004. The respective roles of roots and earthworms in restoring physical properties of vertisol under a *Digitaria decumbens* pasture (Martinique, WI). Agric. Ecosyst. Environ. 103, 343–355. https://doi.org/10.1016/j.agee.2003.12.012.

Boyer, J., Michellon, R., Chabanne, A., Reversat, G., Tibere, R., 1999. Effects of trefoil cover crop and earthworm inoculation on maize crop and soil organisms in Reunion Island. Biol. Fertil. Soils 28, 364–370. https://doi.org/10.1007/s003740050505.

Brais, Bhéreur, P., Gagnon, D., Coderre, D., 1989. Effets de différentes pratiques sylvicoles et de l'introduction de vers de terre sur la structure du sol en plantations de feuillus. Can. J. Soil Sci. 69, 705–709.

Brown, G.G., Benito, N.P., Pasini, A., Sautter, K.D., de F Guimarães, M., Torres, E., 2003. No-tillage greatly increases earthworm populations in Paraná state, Brazil. Pedobiologia 47, 764–771. https://doi.org/10.1078/0031-4056-00256.

Butt, K.R., 1993. Reproduction and growth of three deep-burrowing earthworms (Lumbricidae) in laboratory culture in order to assess production for soil restoration. Biol. Fert. Soils 16, 135–138. https://doi.org/10.1007/BF00369415.

Butt, K.R., 1999. Inoculation of earthworms into reclaimed soils: the UK experience. Land Degrad. Dev. 10, 565–575. https://doi.org/10.1002/(SICI)1099-145X(199911/12)10:6<565::AID-LDR356>3.0.CO;2-K.

Butt, K.R., 2008. Earthworms in soil restoration: lessons learned from United Kingdom case studies of land reclamation. Restor. Ecol. 16, 637–641. https://doi.org/10.1111/j.1526-100X.2008.00483.x.

Butt, K. R., 2011. The earthworm inoculation unit technique: development and use in soil improvement over two decades. In a. Karaca (Ed), biology of earthworms. Soil biology, vol. 24, springer, pp. 87-105. doi:https://doi.org/10.1007/978-3-642-14636-7_6.

Butt, K.R., Frederickson, J., Morris, R.M., 1995. An earthworm cultivation and soil inoculation technique for land restoration. Ecol. Eng. 4, 1–9. https://doi.org/10.1016/0925-8574(93)E0053-S.

Butt, K.R., Frederickson, J., Morris, R.M., 1997. The earthworm inoculation unit technique: an integrated system for cultivation and soil-inoculation of earthworms. Soil Biol. Biochem. 29, 251–257. https://doi.org/10.1016/S0038-0717(96)00053-3.

Butt, K.R., Frederickson, J., Lowe, C.N., 1999. Colonisation, survival and spread of earthworms on a partially restored landfill site. Pedobiologia 43, 684–690.

Butt, K.R., Lowe, C.N., Frederickson, J., Moffat, A.J., 2004. The development of sustainable earthworm populations at Calvert landfill site. UK. Land Degrad. Dev. 15, 27–36. https://doi.org/10.1002/ldr.585.

Calatrava, J., Barberá, G.G., Castillo, V.M., 2011. Farming practices and policy measures for agricultural soil conservation in semi-arid Mediterranean areas: the case of the Guadalentín basin in Southeast Spain. Land Degrad. Dev. 22, 58–69. https://doi.org/10.1002/ldr.1013.

Capowiez, Y., Bottinelli, N., Sammartino, S., Michel, E., Jouquet, P., 2015. Morphological and functional characterisation of the burrow systems of six earthworm species (Lumbricidae). Biol. Fertil. Soils 51, 869–877. https://doi.org/10.1007/s00374-015-1036-x.

Chantre, E., Cardona, A., 2014. Trajectories of French field crop farmers moving toward sustainable farming practices: change, learning, and links with the advisory services. Agroecol. Sustain. Food Syst. 38, 573–602. https://doi.org/10.1080/21683565.2013.876483.

Curry, J.P., Boyle, K.E., 1995. Restoring soil fauna in reclaimed land, with particular reference to earthworms in cutover peat. Acta Zool. Fennica 196, 371–375.

de Valença, A.W., Vanek, S.J., Meza, K., Ccanto, R., Olivera, E., Scurrah, M., Lantinga, E. A., Fonte, S.J., 2017. Land use as a driver of soil fertility and biodiversity across an agricultural landscape in the central Peruvian Andes. Ecol. Appl. 27, 1138–1154.

Derouard, L., Tondoh, J., Vilcosqui, L., Lavelle, P., 1997. Effects of earthworm introduction on soil processes and plant growth. Soil Biol. Biochem. 29, 541–545. https://doi.org/10.1016/S0038-0717(96)00035-1.

Edwards, C.A., Lofty, J.R., 1980. Effects of earthworm inoculation upon the root growth of direct drilled cereals. J. Appl. Ecol. 17, 533. https://doi.org/10.2307/2402635.

FAO and ITPS, 2015. Status of the World's Soil Resources (SWSR) – Main Report. Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils, Rome, Italy.

Forey, E., Chauvat, M., Coulibaly, S.F.M., Langlois, E., Barot, S., Clause, J., 2018. Inoculation of an ecosystem engineer (earthworm: *Lumbricus terrestris*) during experimental grassland restoration: consequences for above and belowground soil compartments. Appl. Soil Ecol. 125, 148–155. https://doi.org/10.1016/j.apsoil.2017.12.021.

Frazão, J., De Goede, R.G.M., Salánki, T.E., Brussaard, L., Faber, J.H., Hedde, M., Pulleman, M.M., 2019. Responses of earthworm communities to crop residue management after inoculation of the earthworm *Lumbricus terrestris* (Linnaeus, 1758). Appl. Soil Ecol. 142, 177–188. https://doi.org/10.1016/j.apsoil.2019.04.022.

Furuya-Kanamori, L., Barendregt, J.J., Doi, S.A.R., 2018. A new improved graphical and quantitative method for detecting bias in meta-analysis. Int. J. Evid. Based Healthc. 16, 195–203. https://doi.org/10.1097/XEB.0000000000000141.

Garnsey, R.B., 1994. The Ecology and Management of Lumbricid Earthworms in the Midlands of Tasmania. PhD thesis,. University of Tasmania, Australia.

Gilot, C., 1997. Effects of a tropical geophageous earthworm, *M. anomala* (Megascolecidae), on soil characteristics and production of a yam crop in Ivory Coast. Soil Biol. Biochem. 29, 353–359. https://doi.org/10.1016/S0038-0717(96)00046-6.

Gilot-Villenave, C., Lavelle, P., Ganry, F., 1996. Effects of a tropical geophagous earthworm, *Millsonia anomala*, on some soil characteristics, on maize-residue decomposition and on maize production in Ivory Coast. Appl. Soil Ecol. 4, 201–211. https://doi.org/10.1016/S0929-1393(96)00119-9.

Golli, S., 2015. Effets de l'inoculation des vers de terre sur la production du maïs : expérimentations en milieux paysans. PhD Thesis,. Université Nangui Abrogoua, Yvory Coast. https://doi.org/10.13140/RG.2.1.4237.5123.

Gregory, A.S., Ritz, K., McGrath, S.P., Quinton, J.N., Goulding, K.W.T., Jones, R.J.A., Harris, J.A., Bol, R., Wallace, P., Pilgrim, E.S., Whitmore, A.P., 2015. A review of the impacts of degradation threats on soil properties in the UK. Soil Use Manage. 31, 1–15. https://doi.org/10.1111/sum.12212.

Hedges, L.V., Olkin, I., 1985. Parametric Estimation of Effect Size from a Series of Experiments. Statistical Methods for Meta-Analysis. Elsevier, in, pp. 107–145. https://doi.org/10.1016/B978-0-08-057065-5.50011-7.

Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The Meta-analysis of response ratios in experimental ecology. Ecology 80, 1150–1156. doi:https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2.

Hoeffner, K., Monard, C., Cluzeau, D., Santonja, M., 2019. Response of temperate anecic earthworm individual biomass to species interactions. Appl. Soil Ecol. 144, 8–11. https://doi.org/10.1016/j.apsoil.2019.06.017.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69, 373–386. https://doi.org/10.2307/3545850.

Lavelle, P., Martin, A., 1992. Small-scale and large-scale effects of endogeic earthworms on soil organic matter dynamics in soils of the humid tropics. Soil Biol. Biochem. 24, 1491–1498. https://doi.org/10.1016/0038-0717(92)90138-N.

Lemtiri, A., Colinet, G., Alabi, T., Cluzeau, D., Zirbes, L., Haubruge, É., Francis, F., 2014. Impacts of earthworms on soil components and dynamics. A review. Biotechnol. Agron. Soc. Environ. 18, 121–123.

Li, Y., Wang, J., Shao, M., 2021. Assessment of earthworms as an indicator of soil degradation: a case-study on loess soils. Land Degrad. Dev. 32, 2606–2617. https://doi.org/10.1002/ldr.3928.

Liu, T., Chen, X., Gong, X., Lubbers, I.M., Jiang, Y., Feng, W., Li, X., Whalen, J.K., Bonkowski, M., Griffiths, B.S., Hu, F., Liu, M., 2019. Earthworms coordinate soil biota to improve multiple ecosystem functions. Curr. Biol. 29, 3420–3429. https://doi.org/10.1016/j.cub.2019.08.045.

Marashi, A.R.A., Scullion, J., 2004. Porosity and hydrological changes in surface mine soils. In: ISCO – 13th International Soil Conservation Organisation Conference. Conserving Soil and Water for Society: Sharing Solutions, 405. Brisbane.

McLean, M.A., Parkinson, D., 2000. Introduction of the epigeic earthworm *Dendrobaena octaedra* changes the oribatid community and microarthropod abundances in a pine forest. Soil Biol. Biochem. 32, 1671–1681. https://doi.org/10.1016/S0038-0717(00)00083-3.

Medina-Sauza, R.M., Álvarez-Jiménez, M., Delhal, A., Reverchon, F., Blouin, M., Guerrero-Analco, J.A., Cerdán, C.R., Guevara, R., Villain, L., Barois, I., 2019. Earthworms building up soil microbiota, a review. Front. Environ. Sci. 7, 81. https://doi.org/10.3389/fenvs.2019.00081.

Müller, P., Neuhoff, D., Nabel, M., Schiffers, K., Döring, T.F., 2022. Tillage effects on ground beetles in temperate climates: a review. Agron. Sustain. Dev. 42, 65. https://doi.org/10.1007/s13593-022-00803-6.

Nielson, R.L., 1951. Earthworms and soil fertility. J. N. Z. Grassl. 13, 158–167.

Nuutinen, V., Butt, K.R., Jauhiainen, L., 2011. Field margins and management affect settlement and spread of an introduced dew-worm (*Lumbricus terrestris* L.) population. Pedobiologia 54, S167–S172. https://doi.org/10.1016/j.pedobi.2011.07.010.

Orwin, R.G., 1983. A fail-safe N for effect size in Meta-analysis. J. Educ. Stat. 8, 157. https://doi.org/10.2307/1164923.

Pérès, G., Cluzeau, D., Curmi, P., Hallaire, V., 1998. Earthworm activity and soil structure changes due to organic enrichments in vineyard systems. Biol. Fertil. Soils 27, 417–424. https://doi.org/10.1007/s003740050452.

Piearce, T.G., Budd, T., Hayhoe, J.M., Sleep, D., Clasper, P.J., 2003. Earthworms of a land restoration site treated with paper mill sludge. Pedobiologia 47, 792–795. https://doi.org/10.1078/0031-4056-00260.

Ramade, F., 2020. Introduction à l'écologie de la conservation : La protection de la nature pour une humanité durable. Lavoisier.

Ratsiatosika, O., Razafindrakoto, M., Razafimbelo, T., Rabenarivo, M., Becquer, T., Bernard, L., Trap, J., Blanchart, E., 2021. Earthworm inoculation improves upland Rice crop yield and other Agrosystem Services in Madagascar. Agriculture 11, 60. https://doi.org/10.3390/agriculture11010060.

Robinson, C.H., Piearce, T.G., Ineson, P., Dickson, D.A., Nys, C., 1992. Earthworm communities of limed coniferous soils: field observations and implications for forest management. For. Ecol. Manage. 55, 117–134. https://doi.org/10.1016/0378-1127(92)90096-R.

Rockwell, S., Kimler, B.F., Moulder, J.E., 2006. Publishing negative results: the problem of publication Bias. Radiat. Res. 165, 623–625. https://doi.org/10.1667/RR3573.1.

Rodríguez, M.P., Domínguez, A., Moreira Ferroni, M., Wall, L.G., Bedano, J.C., 2020. The diversification and intensification of crop rotations under no-till promote earthworm abundance and biomass. Agronomy 10, 919. https://doi.org/10.3390/agronomy10070919.

Sachet, E., Mertz, O., Le Coq, J.-F., Cruz-Garcia, G.S., Francesconi, W., Bonin, M., Quintero, M., 2021. Agroecological transitions: a systematic review of research

approaches and prospects for participatory action methods. Front. Sustain. Food Syst. 5, 709401. https://doi.org/10.3389/fsufs.2021.709401.
Schmidt, O., Curry, J.P., Hackett, R.A., Purvis, G., Clements, R.O., 2001. Earthworm communities in conventional wheat monocropping and low-input wheat-clover intercropping systems. Ann. Appl. Biol. 138, 377–388. https://doi.org/10.1111/j.1744-7348.2001.tb00123.x.
Scholes, R., Brainich, A., Barger, N., Cantele, M., Gardner, T., Holland, T.G., Kohler, F., Kotiaho, J.S., Maltitz, G.V., 2018. Summary for Policymakers of the Thematic Assessment Report on Land Degradation and Restoration of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.
Schon, N.L., Mackay, A.D., Gray, R.A., Dodd, M.B., 2014. The action of an anecic earthworm (*Aporrectodea longa*) on vertical soil carbon distribution in New Zealand pastures several decades after their introduction. Eur. J. Soil Biol. 62, 101–104. https://doi.org/10.1016/j.ejsobi.2014.03.002.
Stein, A., Bekker, R.M., Blom, J.H.C., Rogaar, H., 1992. Spatial variability of earthworm populations in a permanent polder grassland. Biol. Fert. Soils 14, 260–266. https://doi.org/10.1007/BF00395461.
Tondoh, J.E., 2006. Seasonal changes in earthworm diversity and community structure in Central Côte d'Ivoire. Eur. J. Soil Biol. 42, S334–S340. https://doi.org/10.1016/j.ejsobi.2006.09.003.
Uvarov, A.V., 2009. Inter- and intraspecific interactions in lumbricid earthworms: their role for earthworm performance and ecosystem functioning. Pedobiologia 53, 1–27. https://doi.org/10.1016/j.pedobi.2009.05.001.
van der Werff, P.A., Noordhuis, R., Dekkers, Th.B.M., 1998. Introduction of earthworms into an organic arable farming system. Appl. Soil Ecol. 9, 311–317. https://doi.org/10.1016/S0929-1393(98)00082-1.
van Groenigen, J.W., Lubbers, I.M., Vos, H.M.J., Brown, G.G., De Deyn, G.B., van Groenigen, K.J., 2015. Earthworms increase plant production: a meta-analysis. Sci. Rep. 4, 6365. https://doi.org/10.1038/srep06365.
van Rhee, J.A., 1969. Inoculation of earthworms in a newly drained polder. Pedobiologia 9, 128–132.
Wever, L.A., Lysyk, T.J., Clapperton, M.J., 2001. The influence of soil moisture and temperature on the survival, aestivation, growth and development of juvenile *Aporrectodea tuberculata* (Eisen) (Lumbricidae). Pedobiologia 45, 121–133. https://doi.org/10.1078/0031-4056-00074.
Wu, P., Zhang, H., Cui, L., Wickings, K., Fu, S., Wang, C., 2017. Impacts of alpine wetland degradation on the composition, diversity and trophic structure of soil nematodes on the Qinghai-Tibetan plateau. Sci. Rep. 7, 837. https://doi.org/10.1038/s41598-017-00805-5.
Xiao, Z., Wang, X., Koricheva, J., Kergunteuil, A., Le Bayon, R., Liu, M., Hu, F., Rasmann, S., 2018. Earthworms affect plant growth and resistance against herbivores: a meta-analysis. Funct. Ecol. 32, 150–160. https://doi.org/10.1111/1365-2435.12969.